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Nitrogen uptake and coupled nitrification-denitrification in riverine sediments with benthic microalgae and rooted macrophytes

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Abstract:	We measured benthic fluxes of dissolved inorganic carbon, ammonium, nitrate and coupled nitrification-denitrification in fluvial sediments with benthic microalgae and submerged macrophytes (Vallisneria spiralis L.). Two sites with different water column nitrate concentration and sediment organic content were investigated. We hypothesized that: a) nitrate availability promotes water column nitrogen uptake and attenuates primary producers-bacteria competition; b) coupled nitrification-denitrification is stimulated by radial oxygen loss; c) macrophyte meadows favour nitrogen retention and permanent loss. In March, July and October 2008 microcosms containing sediments with benthic algae and macrophytes were incubated in the light and in the dark for inorganic carbon and nitrogen flux measurement. Coupled nitrification-denitrification rates were determined via 15NH4+ injection in the pore water and quantification of the produced 29N2 and 30N2. Sediments with V. spiralis were mostly autotrophic, ammonium sink and displayed higher coupled nitrification-denitrification rates compared to sediments with microphytobenthos. Highest rates, up to 100 µmol N m-2h-1, were measured at the more eutrophic site and in the light. Macrophyte theoretical nitrogen requirements and measured dissolved inorganic nitrogen fluxes suggest a shift from root to leaf- uptake at the nitrate-rich site. We speculate that light-dependent radial oxygen loss by V. spiralis counteracts the reduced chemical environment in organic-rich sediments and promotes the coupling of ammonification, nitrification and denitrification in the rhizosphere. Higher leaf uptake of inorganic nitrogen at the nitrate-rich site may attenuate roots-bacteria competition for nitrogen and favour nitrogen dissipation via denitrification.
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Nitrogen uptake and coupled nitrification-denitrification in riverine sediments with benthic microalgae and rooted macrophytes

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Abstract

We measured benthic fluxes of dissolved inorganic carbon, ammonium, nitrate and coupled nitrification-denitrification in fluvial sediments with benthic microalgae and submerged macrophytes (*Vallisneria spiralis* L.). Two sites with different water column nitrate concentration and sediment organic content were investigated. We hypothesized that: a) nitrate availability promotes water column nitrogen uptake and attenuates primary producers-bacteria competition; b) coupled nitrification-denitrification is stimulated by radial oxygen loss; c) macrophyte meadows favour nitrogen retention and permanent loss. In March, July and October 2008 microcosms containing sediments with benthic algae and macrophytes were incubated in the light and in the dark for inorganic carbon and nitrogen flux measurement. Coupled nitrification-denitrification rates were determined via $^{15}NH_4^+$ injection in the pore water and quantification of the produced $^{29}N_2$ and $^{30}N_2$. Sediments with *V. spiralis* were mostly autotrophic, ammonium sink and displayed higher

coupled nitrification-denitrification rates compared to sediments with microphytobenthos. Highest rates, up to 100 µmol N $m^{-2}h^{-1}$, were measured at the more eutrophic site and in the light.

Macrophyte theoretical nitrogen requirements and measured dissolved inorganic nitrogen fluxes suggest a shift from root to leaf- uptake at the nitrate-rich site. We speculate that light-dependent radial oxygen loss by *V. spiralis* counteracts the reduced chemical environment in organic-rich sediments and promotes the coupling of ammonification, nitrification and denitrification in the rhizosphere. Higher leaf uptake of inorganic nitrogen at the nitrate-rich site may attenuate roots-bacteria competition for nitrogen and favour nitrogen dissipation via denitrification.

Introduction

In illuminated sediments, the activity of primary producers may regulate various processes of benthic nitrogen (N) cycling (Risgaard-Petersen et al. 2003; Tyler et al. 2003; McGlathery et al. 2007; Nizzoli et al. 2014; Soana et al. 2015; Decleyre et al. 2015). Autotrophic sediments with microphytobenthos (MPB) are effective filters for inorganic N, preventing its release to the water column mainly via uptake at the interface (Bartoli et al. 2003; Tyler et al. 2003; Sundbäck et al. 2004). MPB may translocate and retain N within the mat and inhibit the activity of N-related microbial communities (Risgaard-Petersen et al. 2003). Underlying mechanisms include pore water pH and O_2 variations induced by photosynthesis at the interface, removal of pore water ammonium from the upper sediment horizon and production of specific inhibitors of bacterial activity (Risgaard-Petersen et al. 2003). MPB competes effectively for N in oligotrophic environments and tends to minimize its net loss to the water, via recycling, or to the atmosphere, via denitrification or anammox. Uptake processes are therefore quantitatively higher than microbial transformations leading to net N_2 losses, resulting in elevated uptake to denitrification ratios (Sundbäck et al. 2004).

Sediments with submersed aquatic vegetation (SAV) display similar traits, with root uptake as the major benthic N flux (Caffrey and Kemp 1992; Risgaard-Petersen et al. 1998, Soana et al. 2015). The removal of substantial amounts of inorganic N from pore waters regulates diffusive gradients to the water column as well as relevant microbial processes as nitrification, denitrification and nitrogen fixation (Risgaard-Petersen et al. 1998; Sand-Jensen et al. 2005; Racchetti et al. 2010; Soana et al. 2012). Rooted macrophytes may transfer variable oxygen amounts from the roots to the sediment via radial oxygen loss (ROL), to allow cells respiration in an anoxic medium (Laskov et al. 2006; Lemoine et al. 2012). ROL promotes oxic conditions in the rhizosphere that may stimulate the mineralization of organic matter, and therefore ammonification and nitrification, as well as several redox-sensitive biogeochemical processes (Carpenter et al. 1983; Caffrey and Kemp 1992; Risgaard-Petersen and Jensen 1997; Soana et al. 2012).

A high uptake to denitrification ratio is expected also for SAV, as elevated N requirement and assimilation may stimulate N-fixation and outcompete other N-related microbial processes (Risgaard-Petersen and Jensen 1997;

McGlathery et al. 1998). Coupled nitrification-denitrification in the rhizosphere for example is quantitatively small compared to plant uptake and it tends to increase in the dark when assimilation decreases (Reddy et al. 1989; Risgaard-Petersen and Jensen 1997; Risgaard-Petersen et al. 1998; Ottosen et al. 1999; Nicolaisen et al. 2004).

Most studies analysing N cycling in illuminated sediments have explored nutrient-poor lentic environments where primary producers rely on pore water for their N requirements. An interesting question is to verify whether the large dominance of uptake versus coupled nitrification-denitrification persists also under conditions of large inorganic N supply in the water column. Nizzoli et al. (2014) have demonstrated similar rates of assimilation and denitrification in freshwater sediments with benthic vegetation and elevated concentrations of nitrate (NO_3^-) in the water column but in their study they did not considered coupled nitrification-denitrification occurring in the rhizosphere.

At N-rich sites, both MPB and SAV may perform N-assimilation from bottom water, due to much faster advective compared to diffusive nutrient transfer (Stevens and Hurd 1997; Lorenzen et al. 1998; Madsen et al. 2001; Madsen and Cedergreen 2002). Benthic algae are demonstrated to assimilate NO_3^- from the water (Lorenzen et al. 1998) while some freshwater plants are able to increase NO_3^- reductase activity and assimilation by the leaves (Cedergreen and Madsen 2003; Konnerup and Brix 2010). Under such circumstances, roots would support relevant functions as hormone production and plant anchorage (Agami and Waisel 1986; Schutten et al. 2005) and have probably a minor relevance for assimilation. N uptake from the water may attenuate the competition between primary producers and N-related bacteria and may lower the uptake to denitrification ratio. MPB and SAV may in fact stimulate coupled nitrification-denitrification via augmenting the oxic sediment volume (Vartapetian and Jackson 1997; Pezeshki 2001; Racchetti et al. 2010; Soana et al. 2014). We speculate that the ratio between N assimilation and loss via denitrification may vary differentially along eutrophication gradients in sediments with MBP and SAV. The plasticity of rooted plants may in fact result in enhanced ROL to counteract chemically reduced pore water, resulting in much higher oxygen release in deep, ammonium (NH_4^+) rich sediments. This would result in a much larger volume of oxic sediment where nitrification may occur, within an anoxic bulk where the produced nitrate may be denitrified (Wang and Yu 2007; Yu et al. 2010; Soana and Bartoli 2013; Soana et al., 2015).

The aim of the present work was to investigate N assimilation and loss in riverine sediments with MPB and SAV (*Vallisneria spiralis* L., Hydrocharitaceae), under different inorganic N availability. The study area is a lowland sector of the Mincio River (Northern Italy), characterized by illuminated sediments with MPB and SAV (Pinardi et al. 2009; Ribaudo et al. 2011; Bartoli et al. 2012; Bolpagni et al. 2013). Two sites were compared, with the downstream one having higher organic matter content in sediments and NO_3^- in water. We hypothesized that a) NO_3^- availability promotes the uptake of water column N and attenuates primary producers-bacteria competition for N; b) denitrification is more stimulated in sediment with SAV compared to sediments with MPB, in the light compared to dark conditions

and in organic-rich compared to less enriched sediments due to higher ROL and NH_4^+ availability; c) organic-rich riverine sediments with SAV are sites of N retention and permanent loss.

Materials and Methods

The study was carried out in the Mincio River (Northern Italy) at two experimental areas, upstream (M1) and downstream (M2) a wastewater treatment plant (nearly 600,000 equivalent inhabitants in summer). At both sites, muddy sediments with an organic matter content of 6.4±0.2% and 10.6±0.3% at M1 and M2, respectively, hosted monospecific meadows of the submerged macrophyte *Vallisneria spiralis* L. and patches devoid of macrophytic vegetation (Pinardi et al., 2009; Racchetti et al. 2010; Ribaudo et al. 2011).

An approach based on the incubation of microcosms with MPB and SAV under controlled conditions after a 3 weeks acclimatization period was adopted (Ribaudo et al. 2011; Soana et al. 2015). Water, sediments and plants were collected from each site in 3 periods: March, July and October 2008, in order to analyse the whole vegetative period of *V. spiralis*. Surface sediments were collected at M1 and M2 and for each site sediments were immediately sieved, homogenized and transferred into cylindrical Plexiglas microcosms (i.d. 7.5 cm, height 10 cm, wall thickness 0.5 cm, n=16 at each site)(Fig.1). Each microcosm was provided with four series of vertical holes filled with silicon glue and 1 cm spaced. Specimens of *V. spiralis* were carefully collected from the two sites in order to preserve intact the rhizosphere for the transplant. Plants were washed with in situ water and transplanted in 8 microcosms per site while 8 microcosms contained bare sediments (Fig. 1). Experimental plant density (2-3 individuals per microcosm) reflected that measured in situ (500-750 ind m^{-2}, by harvesting 3 replicate frames in each sampling period and at each site). Once created all microcosms, with and without plants, were immediately transferred for 3 weeks on the riverbed under natural temperature, light and flow conditions, half in patches devoid of plants and half within *V. spiralis* meadows. We considered this period as sufficient for the development of microalgal mats on the surface of bare sediments, for the plant to overcome the transplant stress and grow, for the roots to modify the pore water chemical environment, for the bacteria communities adjacent to roots to develop and for the microgradients between pore and bottom water solutes to establish (Racchetti et al. 2010; Ribaudo et al. 2011; Soana et al. 2012 and 2015). Moreover, all microcosms underwent the same processes (i.e. sedimentation) as adjacent natural sediment with MPB or SAV and, once retrieved, they were incubated in the laboratory avoiding root damage, lateral transport of biomass and destruction of sedimentary natural gradients, drawbacks generally occurring during cores collection, in particular within SAV meadows.

During the acclimatization period, water temperature (YSI Multiple Probe, mod. 556) and PAR intensity at the sediment-water interface (Delta OHM, HD9021 model) were measured. At the end of the acclimatization, all microcosms were recovered and transferred underwater into Plexiglass liners (i.d. 8 cm, height 30 cm) provided with a

rubber stopper at the bottom, with no plant and sediment disturbance (Fig. 1). The outer microcosm diameter perfectly fitted the liner inner diameter and after the underwater procedure, intact cores with undisturbed MPB or SAV were obtained. All cores were submerged in coolbags containing site water, and carried to the laboratory within two hours, together with nearly 100 l of site water for preincubation and incubation procedures. In the laboratory, all cores were submerged with the top open in two 50 l incubation tanks, containing vigorously aerated and mixed site water from M1 and M2. All cores were provided with a teflon-coated magnetic bar, driven by an external magnet rotating at 40 rpm. Magnetic bars were fixed in the upper portion of each liner, to avoid sediment resuspension and fronds damage.

At day 2 and day 3 after their recover, all microcosms within the liners underwent two distinct incubations. The first (day 2) targeted dissolved inorganic carbon and dissolved inorganic N fluxes and the second (day 3) targeted coupled nitrification-denitrification rate (detailed methods are reported in the following paragraphs). For each site and for each sampling period, 8 microcosms (4 with MPB and 4 with SAV) were incubated in the light and 8 microcosms (4 with MPB and 4 with SAV) were incubated in the dark (Fig. 1).

Dissolved gas and nutrient fluxes

All flux measurements were performed as short-term batch incubation under continuous water stirring, reproducing in situ temperature and average light conditions (Dalsgaard et al. 2000; Pinardi et al. 2009; Soana et al. 2015). The incubation time (3-6 h) varied seasonally, in order to keep the concentration of dissolved oxygen within ~20-30% of the initial value. Incubations in the light were performed at the average irradiance of each sampling period. Values, measured at the sediment-water interface, were ~300, ~500 and ~200 µE m^{-2} s^{-1} in spring, summer and autumn, respectively. Incubations started when each core was closed at the top with a transparent lid provided with a sampling port and a one-way valve. During the incubation, water samples (~40 ml, corresponding to ~4% of the water volume in the core) were collected 3 times (initial, intermediate, final) at regular time intervals from each sampling port using plastic syringes. An equivalent amount of water was replaced with water from the incubation tank through the one-way valve.

Samples for dissolved inorganic carbon (TCO_2) were transferred to 12 ml Exetainers (Labko, UK) and immediately titrated with 0.1 N HCl (detection limit 1 µM, precision ±5%)(Anderson et al. 1986). Samples for NH_4^+ and NO_3^- determinations were filtered through Whatman GF/F glass fibre filters, transferred to plastic vials and frozen. Within one week NH_4^+ was determined spectrophotometrically using salicylate and hypochlorite in the presence of sodium nitroprussiate (detection limit 0.4 µM, precision ±3%) (Bower and Holm-Hansen 1980). NO_3^- was determined after reduction to nitrite (NO_2^-) in the presence of cadmium and NO_2^- was determined spectrophotometrically using sulphanilamide and N-(1-naphtyl)ethylendiamine (detection limit 0.2 µM, precision ±5%) (Golterman et al. 1978).

Hourly fluxes of TCO_2, NH_4^+ and NO_3^- were calculated by linear regression of concentrations *versus* incubation time and expressed as rates per square meter (mmol or µmol m^{-2} h^{-1}). Positive fluxes are directed from the sediment to the water column while negative fluxes are from the water to the sediment. Daily fluxes were calculated by multiplying light and dark rates by the corresponding number of light and dark hours in each sampling season.

The theoretical N requirement to sustain benthic microalgal and macrophytic primary production was calculated from inorganic carbon fluxes assuming net production equal to TCO_2 fluxes measured in the light and gross production equal to the difference between TCO_2 fluxes measured in the light and in the dark. To this purpose, TCO_2 fluxes measured in the light (only negative values) were divided by C/N ratios of 9 and 13 for MPB and SAV, respectively (Sundback et al. 2004, Racchetti et al. 2010).

Coupled nitrification-denitrification rates

After flux measurements, the top lids were removed and all cores were left submerged in the tanks, renewing the water. The following day, a second incubation was performed to measure coupled nitrification-denitrification rates (DNF_N). All microcosms were removed underwater from the liners and anoxic $^{15}NH_4^+$ solution (10 mM, 98 atom % ^{15}N) was injected into the sediments via glass syringes (Hamilton 725RN 250 µl, ga 22S/51mm/pst 2), through the silicon glue lateral ports (Caffrey and Kemp 1992). The whole 10 cm sediment column was labelled, for a total of 40 injections per microcosm. During each injection, the tracer was distributed homogeneously along the 4 cm needle path. The volume of $^{15}NH_4^+$ solution added to each microcosm varied seasonally. It was calculated in order to enrich by nearly 30% the sediment NH_4^+ pool (pore water + exchangeable NH_4^+). Sediment NH_4^+ pools were measured on in situ sediment samples and varied from ~300 to ~600 µM at M1 and from ~400 to ~1000 µM at M2. Injected volumes of 10 mM $^{15}NH_4^+$ solution varied from 50 to 250 µl, corresponding to a total volume between 2 and 10 ml, over a sediment volume of nearly 400 ml in each microcosm. This procedure took approximately 5 minutes per unit; thereafter each microcosm was transferred into the tank and then underwater into a liner, that was immediately sealed with a bottom stopper and a top lid to start the incubation. Incubation time varied seasonally: 7-9 hours in spring, 4-5 hours in summer and 5-6 hours in autumn. The first (targeting fluxes) and second incubation (targeting coupled nitrification-denitrification rates) were paired so that the same microcosms incubated in the light for fluxes were incubated in the light for DNF_N.

At the end of the incubation 2 mL of 7M $ZnCl_2$ was added to the water phase of each liner and the sediment and water phase were gently slurred. A subsample of the slurry was collected, transferred into 12 mL Exetainers and further poisoned with 200 µl of 7M $ZnCl_2$ to stop bacterial activity. At the end of this procedure each microcosm with *V.*

spiralis was sieved through a 0.2 cm mesh. Aboveground (leaves) and belowground (roots) biomass were separated, gently rinsed with *in situ* water and desiccated at 50 °C until constant weight was reached.

$^{14}N^{15}N$ and $^{15}N^{15}N$ abundance in N_2 was analysed by mass spectrometry at the National Environmental Research Institute, Department of Marine Ecology, Silkeborg (Denmark). DNF_N rate was calculated as the sum of D_{15} and D_{14}, which are the rates of denitrification of $^{15}NO_3^-$ and $^{14}NO_3^-$ produced within the sediments via $^{15}NH_4^+$ and $^{14}NH_4^+$ oxidation, respectively, according to Risgaard-Petersen and Jensen (1997) and Risgaard-Petersen et al. (1998) and the assumptions of the isotope pairing technique (IPT) of Nielsen (1992):

$D_{15} = p(^{15}N^{14}N)+2p(^{15}N^{15}N)$

$D_{14} = p(^{15}N^{14}N) + 2p(^{14}N^{14}N)$

where:

D_{15} = rates of coupled nitrification-denitrification based on $^{15}NO_3^-$ generated by nitrification of added $^{15}NH_4^+$

D_{14} = rates of coupled nitrification-denitrification based on $^{14}NO_3^-$ generated by nitrification of $^{14}NH_4^+$ originally present or produced by ammonification process

$p(^{14}N^{14}N)$, $p(^{15}N^{14}N)$ and $p(^{15}N^{15}N)$ = rates of production of labelled and unlabelled N_2 species.

The $^{15}NH_4^+$ injection method has the limit of the not-homogeneous pore water labelling, compared to the diffusion and perfusion techniques used by Risgaard-Petersen and Jensen (1997), Risgaard-Petersen et al. (1998) and Ottosen et al. (1999) for vegetated sandy sediments. However, these techniques are not suitable for fine grained, muddy sediments as those at the two study sites. Another limit of the adopted method is the possible violation of the IPT assumptions and the risk to underestimate DNF_N due to the presence of multiple hotspots of nitrification and denitrification in the rhizosphere that may determine variable ratios of $^{14}NO_3^-$ and $^{15}NO_3^-$(Risgaard-Petersen and Jensen 1997; Soana et al. 2015).

Statistical analyses

Data analysis was done on seasonal fluxes and separately for light and dark conditions due to demonstrated effects of illumination on primary producers-related processes (Reddy et al. 1989; Risgaard-Petersen and Jensen 1997; Caraco and Cole 2002; Caraco et al. 2006; Lemoine et al. 2012). All comparisons among sampling sites (M1 and M2) and primary producers (MPB and SAV) were done using a two-way analysis of variance (ANOVA). If the effect of the considered factors was significant, pairwise comparisons were performed using the Holm-Sidak test. Sample size was equal in all tests and data were not transformed as they met the assumptions of normality and equal variance (Shapiro-Wilk and Levene's tests). Statistical analyses were run using the program Sigma Plot 13.0 (Systat Software, Inc., CA, USA); statistical significance was set at $p \leq 0.05$. All average values are reported with associated standard error (SE).

Results

TCO_2 fluxes in sediments with MPB and SAV

At M1 the total biomass of *V. spiralis* was rather constant along the three sampling periods, whilst that measured at M2 displayed a summer peak, mostly sustained by the aboveground portion (Table 1). At both sampling sites the minimum root:shoot ratio (~0.17) was recorded in summer. After the transplant and acclimatization period, *V. spiralis* shoots looked healthy, with significant production of new propagules and leaves. A number of ramified oxidized niches around the roots were clearly visible across the microcosm walls (Fig. 1).

Benthic fluxes of TCO_2 measured in the light were always negative in sediments with SAV while they were both negative and positive in sediments with MPB (Table 2). In the dark, sediments were always TCO_2 sources to the water column (Table 2). On a daily basis, sediments with benthic microalgae were a TCO_2 sink only in spring, at M2 (-12.8±8.4 mmol C $m^{-2}d^{-1}$). Sediments with *V. spiralis* were a net daily TCO_2 sink in spring and summer (M2) and in summer and autumn (M1). In the summer, at both sites, TCO_2 uptake peaked with values of ~500 and ~1000 mmol C $m^{-2}d^{-1}$ at M1 and M2, respectively (Table 2).

The analysis of variance suggested that TCO_2 uptake during light incubation was significantly higher in sediments with *V. spiralis* as compared to sediments with MPB, even if differences depended upon the sampling site. In spring, TCO_2 fluxes measured in the light were significantly different between primary producers (two-way ANOVA, $F_{1,12}$=6.068, $p<0.05$) and highest TCO_2 fixation was measured in vegetated sediments at M2 (Holm-Sidak, $p<0.05$). In summer, differences between TCO_2 fluxes measured in the light depended upon the interaction between the factors site and primary producer (two-way ANOVA, $F_{1,12}$=83.113, $p<0.001$). Sediments with SAV, both in M1 and in M2, displayed highest rates of TCO_2 fixation as compared with sediments with MPB (Holm-Sidak, $p<0.001$ for all comparisons) and uptake by *V. spiralis* growing in M2 was higher than that in M1 (Holm-Sidak, $p<0.001$)(Table 2). Also in autumn TCO_2 fluxes measured in the light were dependent on the interaction of the two factors site and primary producers (two-way ANOVA, $F_{1,12}$=63.299, $p<0.001$). Highest TCO_2 fixation was measured in sediment with *V. spiralis* as compared with MPB, and within SAV they were higher in M1 than in M2 (Holm-Sidak, $p<0.001$).

Benthic respiration was significantly higher in sediments vegetated with *V. spiralis* even if such differences depended also on sampling season and site. In spring, dark TCO_2 fluxes depended on the interaction of the factors site and primary producer (2 way ANOVA, $F_{1,12}$=7.699, $p<0.01$) and the highest TCO_2 release was measured in vegetated sediments of M1. In the summer, dark TCO_2 fluxes were different between sampling sites and primary producers (2 way ANOVA, $F_{1,12}$=7.004 and 73.637, respectively, $p<0.01$). Highest benthic respiration reflected highest plant biomass and was measured at M2 in sediments with SAV (Holm-Sidak, $p<0.001$). In autumn dark TCO_2 production

depended only upon the factor primary producer (2 way ANOVA, $F_{1,12}$=15.18, p<0.01) and rates were always higher in SAV versus MPB sediments (Holm-Sidak, p<0.001) while within SAV, no significant differences were found between M1 and M2 (Holm-Sidak, p=0.58).

Rates of gross primary production varied between 0 and 5.31±0.72 mmol C $m^{-2}h^{-1}$ and between 8.54±1.14 and 131.21±3.04 mmol C $m^{-2}h^{-1}$ in sediments with MPB and SAV, respectively.

Theoretical inorganic nitrogen uptake in sediments with MPB and SAV

The inorganic nitrogen (DIN) requirements to sustain MPB or SAV primary production was calculated from net (UPT_N, light incubations) and gross (UPT_G, light-dark) TCO_2 fluxes; only negative data, meaning TCO_2 consumption from the water column, were used (Table 2). As fluxes in the light include the community respiration of the benthic system, net uptake from these data may underestimate true DIN uptake by both primary producers. The latter is probably within the net and gross UPT, that set lower and upper limits of inorganic nitrogen incorporation, respectively. Calculated net DIN uptake rates by sediments with MPB and SAV have the same pattern (and statistics) of TCO_2 fluxes as they are calculated dividing net and gross TCO_2 fixation in the light by a C/N ratios of 9 and 13 for MPB and SAV, respectively (Table 2).

Benthic microalgae had a scarce relevance as DIN sinks at station M1 in summer and autumn, with a significant uptake calculated only in spring (from 222 to 590 µmol N $m^{-2}h^{-1}$). At M2, the activity of MPB was relevant in all seasons, with calculated DIN uptake between 146 and 957 µmol N $m^{-2}h^{-1}$. Calculated DIN uptake by SAV varied between 287 and 5943 µmol N $m^{-2}h^{-1}$ at M1 and between 280 and 10094 µmol N $m^{-2}h^{-1}$ at M2.

Inorganic nitrogen fluxes in sediments with MPB and SAV

In spring and autumn NH_4^+ fluxes measured in the light were different between sites and primary producers but differences depended upon the interaction between factors (two way ANOVA, $F_{1,12}$=16.96 and 192.12, respectively, p<0.001). In spring ammonium uptake was similar in sediments with MPB while it was higher at M2 in sediments with *V. spiralis* (Holm-Sidak, p<0.001). In autumn, NH_4^+ uptake in sediments with MPB was higher at M2 while in sediments with SAV it was higher in M1 (Holm-Sidak, p<0.001). When comparing sediments with MPB and SAV, higher NH_4^+ uptake was measured in SAV sediments only at M1 while rates were similar at M2 (Holm-Sidak, p<0.001). In summer NH_4^+ uptake was higher in *V. spiralis* vegetated sediments as compared to sediments with MPB (two way ANOVA, $F_{1,12}$=99.09, p<0.001) and, within SAV, in M2 than in M1 (Holm-Sidak, p<0.05). NH_4^+ consumption in the light peaked with over 1000 µmol N $m^{-2}h^{-1}$ (Figs. 2 and 3).

In the dark, differences between ammonium fluxes depended always upon the interaction between primary producers and sampling site (two way ANOVA, $F_{1,12}$=22.09, 132.57 and 11.24 in spring, summer and autumn, respectively, $p<0.01$) (Figs. 2 and 3). In spring dark ammonium fluxes were generally low (within ±50 µmol N $m^{-2}h^{-1}$) and either directed to the water column or to the sediments (Figs. 2 and 3). In the summer, sediments at M1 net regenerated ammonium with a peak measured in the presence of *V. spiralis* (~2000 µmol N $m^{-2}h^{-1}$) (Holm-Sidak, $p<0.001$). At M2 ammonium was net retained in sediments with SAV and net regenerated in the presence of MPB (Figs. 2 and 3). In autumn, at M1, sediments with *V. spiralis* net retained while sediments with MBP net released NH_4^+ to the water column (Holm-Sidak, $p<0.01$). At M2 on the contrary NH_4^+ fluxes were not significantly different from zero and similar in the presence of the two primary producer forms (Holm-Sidak, $p=0.34$).

On a daily basis sediments at M1 were a sink for NH_4^+ in spring and autumn, due to MPB (-1.53±0.35 mmol N $m^{-2}d^{-1}$) and to *V. spiralis* (-7.08±1.12 mmol N $m^{-2}d^{-1}$), respectively. They were net NH_4^+ sources in the summer with comparable rates (3.44±2.92 and 4.04±0.94 mmol N $m^{-2}d^{-1}$ for SAV and MPB, respectively). At M2, sediments with *V. spiralis* were always retaining NH_4^+, with a summer maximum of -30.62±6.59 mmol N $m^{-2}d^{-1}$. Sediments with benthic algae were also a NH_4^+ sink, but only in spring and autumn, while they were a source to the water column in summer (2.20±0.23 mmol N $m^{-2}d^{-1}$).

Fluxes of NO_3^- were mostly directed to the benthic system, with significant differences between sites and rates higher at M2 due to larger availability of water column nitrate (two way ANOVA, $F_{1,12}$=10.78, 3.41 and 212.23 in spring, $p<0.05$, summer, $p=0.09$ and autumn, $p<0.001$, respectively)(Table 1, Figs. 2 and 3). In autumn differences between sites depended also upon the primary producer form (two way ANOVA, $F_{1,12}$=1090.06 $p<0.001$). In spring light fluxes of NO_3^- were all negative, and peaking at M2 in sediments with MPB (Holm-Sidak, $p<0.01$)(Figs. 2 and 3). In summer the picture was similar, with NO_3^- uptake prevailing in all conditions, higher at M2 as compared to M1 and, within M2, similar between MPB and SAV (Holm-Sidak, $p>0.05$)(Figs. 2 and 3). In autumn light fluxes of NO_3^- were negligible at M1 in sediments with both primary producer forms while at M2 they were significantly different with net uptake in SAV and net regeneration in MPB (Holm-Sidak, $p<0.001$)(Figs. 2 and 3).

Dark nitrate fluxes were significantly different between sites only in spring (two way ANOVA, $F_{1,12}$=10.36 $p<0.01$) while in the summer and autumn differences depended upon the interactions between sites and primary producers (two way ANOVA, $F_{1,12}$=47.0 and 3740.30, respectively, $p<0.001$). In spring dark NO_3^- fluxes were different, within sites, in the presence of MPB and SAV (Holm-Sidak, $p<0.05$). In the summer the benthic demand of NO_3^- peaked at M2 in sediments with *V. spiralis* (Holm-Sidak, $p<0.001$) with rates >1000 µmol N $m^{-2}h^{-1}$ while in autumn a similar uptake was measured at the same station in sediments with benthic algae (Figs. 2 and 3). On a daily basis, nitrate production and consumption processes at M1 were nearly balanced and varied between -0.72±1.40 and 1.13±0.86 mmol N $m^{-2}d^{-1}$

measured in spring and in summer in sediments with SAV. At M2, daily NO_3^- fluxes were on the contrary always negative, regardless the primary producer forms, suggesting the dominance of consumption processes. Rates varied between -5.35±1.14 and -14.30±3.60 mmol N $m^{-2}d^{-1}$, measured in spring and summer in sediments with SAV (Figs. 2 and 3).

On a daily basis, sediments with SAV were a net DIN sink in spring and autumn (-2.03±0.71 and -7.29±0.53 mmol N m^{-2} d^{-1}) and a net DIN source (4.57±2.28 mmol N m^{-2} d^{-1}) in summer at M1, while they were always a DIN sink at M2, with a summer peak of -44.91±4.56 mmol N m^{-2} d^{-1} (Figs. 2 and 3). Sediments with benthic algae were a net DIN source in two out of three sampling periods at M1, with the highest regeneration measured in summer and driven by NH_4^+ recycling (4.00±1.05 mmol N m^{-2} d^{-1}). At M2 on the contrary DIN fluxes were always negative and mostly driven by NO_3^- uptake in all seasons. Similar spring and autumn DIN consumption (-7.16±1.28 and -7.65±0.58 mmol N m^{-2} d^{-1}, respectively) were attenuated in summer (-0.85±1.12 mmol N m^{-2} d^{-1}). Overall, striking differences between daily fluxes of DIN in sediments with MPB and SAV were measured only at M2, in the summer period. Here, N demand in sediments with *V. spiralis* was nearly 50 times higher than that in sediments with benthic algae (Figs. 2 and 3).

Nitrification-coupled denitrification in sediments with MPB and SAV

In all seasons rates of DNF_N measured in the light were higher at M2 as compared to M1, regardless the primary producer form (two way ANOVA, $F_{1,12}$=11.50, 5.59 and 24.27 in spring $p<0.01$, summer $p<0.05$ and autumn $p<0.001$, respectively) (Fig. 4). In spring and autumn DNF_N in the light was higher in sediment with SAV (two way ANOVA, $F_{1,12}$=51.43 and 58.08, $p<0.001$), while in summer the differences between primary producer forms were almost significant (two way ANOVA, $F_{1,12}$=3.86, $p=0.07$). In spring, light DNF_N rates measured in sediment with SAV were higher at M2 as compared to M1 (Holm-Sidak, $p<0.01$), while rates measured in sediment with MPB were similar between sites (Holm-Sidak, $p=0.324$). In autumn, light DNF_N rates were higher in sediment with SAV compared to sediment with MPB for both sites (Holm-Sidak, $p<0.001$) and were higher at site M2 as compared to M1 for both primary producers (Holm-Sidak, $p<0.05$).

In spring, differences between dark DNF_N rates depended upon the interaction between primary producers and sampling site (two way ANOVA, $F_{1,12}$=21.86, $p<0.001$). N removal measured in the dark was higher at M2 for both sediments colonized by SAV and MPB (Holm-Sidak, $p<0.001$) and only at M2 rates were higher in sediment with SAV compared to sediment with MPB (Holm-Sidak, $p<0.001$). In summer, DNF_N varied from 16±2 up to 31±5 µmol N m^{-2} h^{-1} in sediment with MPB at M2 and in sediment with SAV at M2, respectively. Rates were similar between sites while differences between primary producers were almost significant (two way ANOVA, $F_{1,12}$=4.14, $p= 0.06$). In autumn,

DNF_N rates depended only upon the factor primary producers (two way ANOVA, $F_{1,12}=17.17$, $p<0.01$) and N removal measured in the dark was higher in sediment with SAV at both sites (Holm-Sidak, $p<0.05$).

On a daily basis, at both sites, DNF_N removed more N in sediments with SAV than in sediments with MPB, with a peak of 1.73± 0.23 mmol N $m^{-2}d^{-1}$ measured in spring at M2. At M1, daily N removal in sediment with SAV was similar among seasons (0.73±0.07, 0.74±0.14 and 0.91±0.07 mmol N $m^{-2}d^{-1}$ for spring, summer and autumn, respectively) whilst in sediments with MPB they increased in the summer with 0.40±0.02 mmol N $m^{-2}d^{-1}$. Also at M2 N removal via DNF_N peaked in the summer in sediment with MPB (0.76±0.02 mmol N $m^{-2}d^{-1}$) whilst in the same season it was minimum in sediments with SAV (1.06±0.23 mmol N $m^{-2}d^{-1}$) if compared with daily rates measured in spring and autumn (1.73±0.23 and 1.51±0.19 mmol N $m^{-2}d^{-1}$, respectively).

Discussion

N cycling in riverine sediments

This study contributes to our understanding of benthic N pathways in illuminated riverine sediments. The relevance of primary producers for benthic N cycling has been extensively studied in lentic and coastal waters, while there are comparatively fewer studies in lotic systems (Pinardi et al. 2009; Desmet et al. 2011; Forshay and Dodson 2011; Soana et al. 2015). Results suggest that across the whole vegetative period and under low and high inorganic nitrogen availability sediments with SAV displayed higher N temporary or permanent removal as compared to sediments with benthic MPB, due to higher rates of primary production, inorganic nitrogen uptake and loss via coupled nitrification-denitrification.

Daily budgets of inorganic carbon revealed in the three sampling periods and at both sites a substantial equilibrium or a net TCO_2 production in excess to fixation in sediments with MBP, suggesting that benthic respiration exceeded photosynthesis by microalgae. Daily budgets of inorganic nitrogen were only partially coupled to those of inorganic carbon as at M1 they were mostly positive, with the prevalence of DIN recycling, while at M2 they were negative, suggesting the dominance of DIN-consuming processes. As at M2 calculated DIN uptake by benthic microalgae was low and most of the DIN daily budget was driven by nitrate consumption, we speculate in these heterotrophic sediments elevated rates of denitrification of water column nitrate (Pinardi et al. 2009; Racchetti et al. 2011; Soana et al. 2015). Such results, for sediments with benthic microalgae, conform to the general finding that autotrophic systems display DIN retention and limited N loss via denitrification while heterotrophic sediments display net DIN recycling and elevated loss via denitrification (Risgaard-Pedersen 2003).

In sediments with SAV inorganic C budgets were negative in 2 out of 3 sampling periods at both sites, suggesting a prevailing net autotrophy and elevated DIN requirements to sustain primary production, in particular in the summer

period. At M1, sediments with *V. spiralis* displayed a reduced release or a net daily uptake of DIN as compared to sediments with MPB, while at M2 *V. spiralis* primary production resulted in negative DIN budgets in all sampling periods.

We analysed comparatively the fluxes of ammonium and nitrate measured during light incubations with calculated DIN requirements by primary producers; calculations were possible only with negative TCO_2, NH_4^+ and NO_3^- net fluxes (Table 3). In particular, we calculated the percentage of theoretical net and gross DIN uptake accounted for by the net NH_4^+ and NO_3^- fluxes measured in the light. With some limitations, such calculation may approximate the fraction of DIN requirements by primary producers sustained by the water column, and by difference it allows to infer that sustained by pore water. Reliable calculations, in sediments with MPB, were done only in spring at both sites. At M1 they suggested that water column supplied between 17 and 44% of the theoretical N demand, mostly as ammonium, while nitrate uptake was irrelevant. At M2 on the contrary DIN fluxes were in large excess to benthic algael uptake; ammonium fluxes satisfied from 33 to 55% of the N demand while nitrate fluxes from 100 to 158% (Table 3). At this site, fluxes of nitrate higher than gross theoretical N demand suggest alternative paths of N consumption as denitrification (Soana et al. 2015).

Similar outcomes resulted from calculations done in sediments with SAV, that were performed in all sampling periods. At M1, DIN fluxes sustained from 12 to 40% of gross and net theoretical N uptake while at M2 such percentage increased, from 19 to 96%. In the summer the share of water column inorganic nitrogen to the plant uptake was minimum, suggesting a major assimilation from pore water. Nitrate contribution was always higher at M2, where concentrations were higher, regardless the sampling period. These results suggest a major relevance of nitrate uptake by the leaves at M2 as compared to M1, sustaining a major fraction of DIN demand by *V. spiralis*. They also suggest higher rates of denitrification of water column nitrate at M2 (Pinardi et al. 2009).

The ratio between coupled nitrification-denitrification and calculated net and gross N uptake was extremely variable in sediments with benthic microalgae, ranging from 0 (when DNF_N rates were undetectable) to incomputable (when sediments were net heterotrophic and uptake was not calculated) (Table 4). In sediments with *V. spiralis*, DNF_N represented a fraction of net and gross N uptake varying from 0.5 to 26.4%. DNF_N was quantitatively irrelevant compared to uptake (<1%) in the summer and at both sampling sites, due to impressive rates of primary production (nearly 520 and 1090 mmol C $m^{-2}d^{-1}$ at M1 and M2, respectively). In spring and autumn, on the contrary, the ration between N lost and that assimilated was relevant and more at M2 than at M1 (Table 4). These results are in agreement with our hypotheses, as at M2, in spring and autumn, water column DIN likely sustained a large fraction of the plant N requirement (Table 3), slowing the competition for pore water N between plants and bacteria.

At M1 and M2, N uptake by the macrophyte represented a major fraction of the total N retained and lost but despite elevated rates of primary production N-related microbial activities in sediments was not depressed. Denitrification associated with the rhizosphere was in fact relevant and nearly two-fold higher downstream as compared with upstream. Furthermore, rates measured in the light, when assimilation peaked, were always higher than those measured in the dark. These results are opposite to those reported in Risgaard Pedersen et al. (1997) for more oligotrophic sediments. We discuss in the following paragraphs how coupled nitrification-denitrification was indirectly supported by *V. spiralis*, an engineering species, through increased ROL and leaf N-uptake.

V. spiralis *root and leaf N uptake*

In oligotrophic systems, rooted plants rely primarily on sediments for assimilation, since benthic mineralization enriches pore water with nutrients while the water column is generally nutrient-limited (Barko et al. 1991; Bedford et al. 1991; Carr and Chambers 1998). However, some macrophytes are demonstrated to maintain root nutrient uptake also under conditions of high nutrient availability in the water column (Thursby and Harlin 1984; Cedergreen and Madsen 2003). Our results suggest a different response of *V. spiralis* to eutrophic conditions. At the downstream site in fact, inorganic N uptake from the water column (leaf assimilation) represented a relevant fraction of inorganic N input to the plant. Our calculations suggest a preferential NH_4^+ uptake by *V. spiralis*, but with a relevant distinction between sites. At the upstream site, water column and regenerated NH_4^+ was the dominant form of inorganic N assimilated by the leaves, while at the downstream site both NH_4^+ and NO_3^- were likely assimilated. At the downstream site, higher availability of water column NO_3^- may stimulate NO_3^- reductase activity, enhancing leaf NO_3^- uptake (Cedergreen and Madsen 2003; Wang et al. 2008; Konnerup and Brix 2010; Takayanagi et al. 2012).

We cannot exclude at the more eutrophic downstream site an inhibitory effect of organic sediments and reduced chemical conditions in the pore water on roots assimilative functions. When growing in organic-rich substrates, submerged macrophyte roots maintain important physiological functions as hormone production and anchorage (Agami and Waisel 1986; Schutten et al. 2005), while they progressively lose other functions as those related to nutrient uptake (Denny 1972; Madsen and Cedergreen 2002). Studies addressing plant morphology suggest that macrophytes growing in eutrophic sites with reduced sediments re-allocate their biomass reducing the belowground portion and augmenting that aboveground. As a consequence, they have a lower root:shoot ratio (RSR) compared with macrophytes growing in oligotrophic systems (Barko and Smart 1986; Van et al. 1999; Madsen and Cedergreen 2002; Xie et al. 2005; Wang and Yu 2007; Li et al. 2012). For example, isoetid species suffer oxygen stress associated with increased sediment organic matter content and they tend to reduce the root biomass, resulting in low RSR. In enriched sediments, isoetid roots become shorter and thicker to minimize the time required by oxygen to reach the apical zones. These plants display low

plasticity and, even under a moderate organic increase, roots may turn atrophic, lose their anchorage and assimilative function and determine the death of the plant (Raun et al. 2010; Pulido et al. 2011). According to Hauxwell et al. (2007) and Pinardi et al. (2009), RSRs measured for *V. spiralis* display a pronounced seasonal variation, with minimum values in summer coinciding with more reduced and hostile chemical condition within sediments. Results from the present study are in agreement with previous findings, as the highest ratio between above and belowground biomass, coinciding with minimum RSR, was determined at both stations in the warmest period (Table 1). However, despite a biomass reduction, the belowground portion of *V. spiralis* appeared healthy and active also in the summer, as suggested by thick halos of light brown sediments all along the root hair length, a proxy of oxidised conditions. Our results, combined with previous findings in the same study area (Ribaudo et al. 2011), suggest an adaptive response of *V. spiralis* to hostile sediment conditions, resulting in root biomass reduction and enhanced radial oxygen loss.

At the upstream site, the flux of inorganic N to the plant was mostly from the pore water, suggesting that roots maintained the assimilation capacity despite biomass reduction. At the NO_3^--rich site, a major part of the inorganic N flux was sustained by the water column, suggesting either a loss of assimilation capacity or enhanced leaf uptake. Regardless the underlying mechanism, any shift from root to leaf uptake attenuates the competition between plants and bacteria for N, with implications for microbially-mediated sediment N processes.

Coupled nitrification-denitrification in the rhizosphere of V. spiralis

Results from this study demonstrate that radial oxygen loss from the roots of *V. spiralis* stimulate coupled nitrification-denitrification. DNF_N rates measured in sediments with SAV were in fact 2 to 6 and 1.5 to 5 fold higher compared with those measured in sediments with MPB at M1 and M2, respectively. Measurements of DNF_N in the rhizosphere of rooted plants were performed in a relatively few other works including marine, brackish and freshwater species (Table 3). Rates measured in the present study are in the range of those reported for submerged plants and the first available for *V. spiralis* colonised sediments (Table 4). However, published studies differ for experimental designs, methods and trophic status of the sites, so direct comparisons should be done with caution. For example, slurry incubations measure potential activity and mass balances provide only indirect measurements, while the $^{15}NH_4^+$ perfusion technique permits direct measurements of DNF_N rates for sites characterized by sandy sediment but not for fine organic sediments (Risgaard-Petersen et al. 1998; Ottosen et al. 1999).

Due to methodological constraints (i.e. not-homogeneous labelling of pore water with $^{15}NH_4^+$) and the occurrence of multiple denitrification zones in the rhizosphere of *V. spiralis*, our estimates of DNF_N are probably underestimated (Reddy et al. 1989; Risgaard-Petersen and Jensen 1997). This suggests that true D_N rates in vegetated sediments are higher than those reported in this work and thus many folds higher than those measured in sediments with benthic

microalgae. The main reason for such large difference is the volume of oxic sediment where nitrification can take place, which augments in the presence of roots because of radial oxygen loss (Hines 2006; Møller and Sand-Jensen 2011; Lemoine et al. 2012). For *V. spiralis* the diffusion of oxygen from the roots to the sediment is not confined to the root tip but visibly occurs along the whole root surface (Fig. 1), resulting in a large net effect for microbial communities and associated processes. DNF_N rates measured in sediments with SAV were different in relation to the sampling period and to the light regime. In a recent paper, Soana and Bartoli (2013) demonstrated that ROL by *V. spiralis* varies on a seasonal basis, with maximum rates estimated in late summer. This was explained in terms of plant plasticity and adaptations to progressively more reduced chemical conditions in the pore water, requiring more oxygen transfer to allow root survival. Seasonal variations of DNF_N are not evident at upstream site, while downstream we measured a summer drop of the process, which is contrary to what described for ROL (Soana and Bartoli 2013). It is likely that in summer the regulation of DNF_N in organic-rich vegetated sediment is complex, with an array of microbial or chemical processes competing with nitrifiers for oxygen and the plant competing with bacteria for nitrogen due to elevated requirements to sustain growth (Sousa et al. 2012).

Higher DNF_N rates in light compared to dark incubations are in agreement with higher ROL during the photosynthetic period, which is expected, but are opposite to what reported in other studies (Risgaard-Petersen and Jensen 1997). Most brackish and marine plants have limited capacity of oxygen transport toward the rhizosphere (Sand-Jensen et al. 1982; Caffrey and Kemp 1991). In marine environments, it is generally believed that oxygen available in the pore water is mostly used to detoxify sediments from the extremely toxic sulphides at the expense of other microbial processes as nitrification, which could explain low rates of DNF_N. In freshwater habitats, studies on coupled nitrification-denitrification were performed on both emergent and submerged plants. Obtained rates are generally higher for emergent macrophytes, likely due to higher ROL, which is in turn dependent upon direct contact of the plant with the atmosphere. Aerenchyma allows the oxygen transport towards the rhizosphere and the oxidation of sediment surrounding roots where aerobic processes can occur. Reddy et al. (1989) measured extremely elevated DNF_N rates in three emergent macrophytes, among the highest reported in the literature (Table 4). Indeed, rates measured in submerged freshwater macrophytes are usually higher for those plants, such as isoetids, that evolve most of the oxygen produced during photosynthesis downwards (Risgaard Pedersen and Jensen 1997; Sand-Jensen et al. 2005; Møller and Sand-Jensen 2012). At oligotrophic sites, nitrogen loss via DNF_N is quantitatively small compared to plant uptake, and it tends to increase in the dark when assimilation decreases (Risgaard-Petersen and Jensen 1997; Risgaard-Petersen et al. 1998). For example, Risgaard-Pedersen and Jensen (1997) reported a ~30% increase of DNF_N rates in sediments with *Lobelia dortmanna* incubated in the dark, suggesting a strong competition between plants and bacteria for N during the photosynthetic period. An interesting outcome of this study is that rates of DNF_N in sediments with *V.*

spiralis were higher in the light (by 7 to 88% and by 29 to 44% at M1 and M2, respectively) compared to dark conditions regardless the sampling season. We speculate that both oxygen and N availability in subsurface sediments can be potentially relevant interrelated factors regulating DNF_N. We address our findings to the increase of oxic volume of sediments where nitrification can occur due to higher ROL in the light, combined with a limited competition between plant and bacteria in a N-rich system. The increasing relevance of leaf to total N uptake and the adaptations that allow *V. spiralis* growth in organic-rich sediments may have a stimulatory effect on subsurface coupled nitrification-denitrification. Leaf uptake weakens root-bacteria competition for inorganic N, while enhanced ROL in a chemically reduced sediment may promote the coupling between ammonification, nitrification and denitrification at the interfaces between the oxic rhizosphere and the surrounding anoxic sediment (Carpenter 1983; Risgaard-Petersen and Jensen 1997; Soana and Bartoli 2013). These results may be plant-specific, as other macrophytes can be less tolerant towards organic enrichment and negatively affected by reduced chemical conditions (Barko and Smart 1986; Raun et al. 2010). Future studies should be extended to other plant species, include other potentially relevant processes for benthic N cycling as N_2 fixation and develop new methodological approaches to measure more precisely DNF_N rates in muddy, organic sediments.

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Table 1 In situ and incubation temperature, $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ concentrations (average ± standard deviation, n=3) and *V. spiralis* biomass (g of dry weight per m^{-2}, average ± standard error, n=8) at the two sites M1 and M2 during spring, summer and autumn experiments

Site	Season	Temperature (°C)	$N\text{-}NO_3^-$ (µM)	$N\text{-}NH_4^+$ (µM)	Aboveground biomass (g_{DW} m^{-2})	Belowground biomass (g_{DW} m^{-2})
M1	Spring	12	13.6 ± 3.4	4.9 ± 0.2	303.4 ± 28.9	193.9 ± 14.3
	Summer	24	3.6 ± 0.2	3.0 ± 0.1	324.3 ± 72.7	55.6 ± 9.8
	Autumn	17	63.7 ± 3.1	6.2 ± 0.2	243.5 ± 37.9	119.8 ± 25.5
M2	Spring	12	75.3 ± 3.6	8.9 ± 0.1	154.7 ± 14.4	126.9 ± 17.2
	Summer	24	66.7 ± 2.1	2.9 ± 0.1	503.4 ± 65.4	85.8 ± 13.4
	Autumn	17	310.5 ± 0.1	2.3 ± 0.6	197.5 ± 33.4	81.4 ± 11.2

Table 2 Benthic fluxes of TCO_2 measured seasonally in the dark (R=community respiration) and in the light (NP=net community production) in microcosms with benthic microalgae (MPB) and submerged aquatic vegetation (SAV). Gross rates (GP=gross community production) were calculated from NP and R. Daily fluxes were calculated by multiplying hourly fluxes by the number of light and dark hours in the different sampling periods and summing the obtained values.

Net (UPT_N) and gross (UPT_G) theoretical nitrogen uptake were calculated dividing NP and GP by the C:N ratios of MPB (9 for benthic microalgae) and SAV (13 for *V. spiralis*), reported by Pinardi et al. (2009) and Racchetti et al. (2010)

			TCO_2 fluxes				**Theoretical DIN uptake**	
Site	**Season**	**Primary producer**	**Dark (R)** mmol m^{-2} h^{-1}	**Light (NP)** mmol m^{-2} h^{-1}	**Gross (GP=NP-R)** mmol m^{-2} h^{-1}	**Daily** mmol m^{-2} d^{-1}	**Net (UPT_N)** µmol m^{-2} h^{-1}	**Gross (UPT_G)** µmol m^{-2} h^{-1}
M1	Spring	MPB	3.31 ± 0.48	-1.99± 0.89	-5.31 ± 0.72	-5.50±17.98	222.0± 99.9	589.5± 79.9
		SAV	11.83 ± 1.82	-3.74 ± 1.53	-15.57 ± 1.68	34.87±31,79	287.5± 117.7	1198.0± 527.8
	Summer	MPB	2.45 ± 0.47	1.76 ± 0.74	-0.70 ± 0.62	48.41±9.86	0	77.3 ± 69.0
		SAV	26.80 ± 6.61	-50.46 ± 2.68	-77.26 ± 5.04	-515.73±68.69	3881.7 ± 206.5	5943.3 ± 1584.5
	Autumn	MPB	0.90 ± 0.31	0.92 ± 0.24	0.02 ± 0.28	21.80±5.41	0	0
		SAV	4.25 ± 0.38	-11.49 ± 1.06	-15.78 ± 0.79	-55.38±15.69	883.7 ± 81.4	1210.6 ± 249.7
M2	Spring	MPB	0.84 ± 0.10	-1.43± 0.56	-2.27 ± 0.40	-12.78±8.45	159.1± 62.4	252.3± 44.9
		SAV	3.95 ± 0.52	-4.93 ± 1.03	-8.88 ± 0.81	-33.94±17.30	379.3 ± 79.1	683.4± 255.9
	Summer	MPB	4.38 ± 0.97	0.35 ± 0.60	-4.03 ± 0.81	38.65±12.37	0	447.6 ± 89.6
		SAV	44.87 ± 3.50	-86.36 ± 2.68	-131.23 ± 3.04	-1088.40±34.59	6643.0 ± 191.6	10094.4 ± 955.5
	Autumn	MPB	1.90 ± 0.31	-1.32 ± 0.41	-3.21 ± 0.36	6.95±6.58	146.3 ± 45.9	356.9 ± 40.5
		SAV	4.91 ± 1.52	-3.64 ± 0.51	-8.54 ± 1.14	15.23±19.54	279.7 ± 39.4	657.1 ± 357.4

Table 3 The fraction of the net (UPT_N) and gross (UPT_G) theoretical nitrogen uptake represented by the benthic fluxes of DIN, $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and by the rates of coupled nitrification-denitrification (DNF_N) is reported. All calculations were performed on processes and rates measured in the light incubations and were limited to negative TCO_2, $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ net fluxes (otherwise the symbol "-" is reported). Calculations were not possible in heterotrophic sediments where N uptake by primary producers was null while DNF_N was measurable; in this case "i" (incomputable) is reported.

Site	**Season**	**Primary producer**	**DIN flux**		**$N\text{-}NH_4^+$ flux**		**$N\text{-}NO_3^-$ flux**		**DNF_N**	
			% UPT_N	% UPT_G	% UPT_N	% UPT_G	% UPT_N	% UPT_G	% UPT_N	% UPT_G
M1	Spring	MPB	44	17	42	16	2	1	0.0	0.0
		SAV	49	12	20	5	29	7	15.0	3.6
	Summer	MPB	-	-	-	-	-	-	i	21.3
		SAV	28	18	27	17	1	0.5	0.9	0.6
	Autumn	MPB	-	-	-	-	-	-	i	i
		SAV	55	40	55	40	0.3	0.2	4.5	3.3
M2	Spring	MPB	211	133	53	33	158	100	7.5	4.8
		SAV	105	59	60	34	45	22	23.0	12.8
	Summer	MPB	7	-	-	-	-	35	i	8.7
		SAV	28	19	24	16	4	3	0.8	0.5
	Autumn	MPB	-	-	66	10	-	-	17.8	2.7
		SAV	237	96	35	15	202	86	26.4	11.2

Table 4 Coupled nitrification-denitrification rates (DNF_N) measured in freshwater and marine vegetated sediments. Employed methods include: A) $^{15}NH_4^+$ injection, B) $^{15}NH_4^+$ perfusion, C) slurry incubation, D) diffusion technique, E) N_2 flux technique, E*) N_2 flux technique with urea injection in the rhyzosphere and F) mass balance. Light (L) or dark (D) incubation conditions are reported. Note: nd indicates values below the detection limit

Macrophyte	Technique	Location	Site	Season	Incubation conditions	DNF_N rate (μmol m^{-2} h^{-1})	References
Vallisneria spiralis (submerged)	A	Italy	River	Spring	L	43 ± 4 (M1) 87 ± 16 (M2)	This study
		Italy	River	Spring	D	5 ± 2 (M1) 49 ± 5 (M2)	
		Italy	River	Summer	L	36 ± 7 (M1) 50 ± 14 (M2)	
		Italy	River	Summer	D	22 ± 9 (M1) 31 ± 5 (M2)	
		Italy	River	Autumn	L	40 ± 5 (M1) 74 ± 3 (M2)	
		Italy	River	Autumn	D	37 ± 4 (M1) 52 ± 16 (M2)	
Littorella uniflora (submerged)	B	Denmark	Lake	-	L	30 ± 8	Ottosen *et al.* (1999)
Potamogeton pectinatus (submerged)	B	Denmark	Lake	-	L	6 ± 3	Ottosen *et al.* (1999)
Potamogeton perfoliatus (submerged)	C	Maryland (USA)	Estuarine pond	Spring	D	638 ± 110	Caffrey & Kemp (1990)
		Maryland (USA)	Estuarine pond	Summer	D	10 ± 1	
		Maryland (USA)	Estuarine pond	Autumn	D	262 ± 15	
Lobelia dortmanna (submerged)	D	Denmark	Lake	Spring	L	25 ± 1	Risgaard-Petersen & Jensen (1997)
	D	Denmark	Lake	Spring	D	35 ± 6	
	B	Denmark	Lake	-	L	25 ± 0.5	Ottosen *et al.* (1999)
	C	Denmark	Lake	-	D	93	
Pontederia cordata (emergent)	A	Florida (USA)	Lake	-	L	247± 36	Reddy *et al.* (1989)
Juncus effusus (emergent)	A	Florida (USA)	Lake	-	L	238± 105	Reddy *et al.* (1989)
Oryza sativa (emergent)	A	Lousiana (USA)	Rice paddy	-	-	284±74	Reddy *et al.* (1989)

	A	Philippines	Lagoon	Autumn	-	117±159	Nicolaisen et al. (2004)
	E	Italy	Rice paddy	-	-	nd	Arth, Frenzel & Conrad (1998)
	E*	Italy	Rice paddy	-	-	343 ± 38	
	B	Denmark	Estuary	-	L	2 ± 0.5	Ottosen *et al.* (1999)
	B	Denmark	Estuary	Spring	L	140 ± 100	
	B	Denmark	Estuary	Spring	D	nd	Risgaard-Petersen *et al.* (1998)
	B	Denmark	Estuary	Summer	L	nd	
	B	Denmark	Estuary	Summer	D	nd	
Zostera marina (submerged) 0	C	Denmark	Estuary	-	D	7 ± 7	Ottosen *et al.* (1999)
	C	Virginia (USA)	Coastal zone	Spring	D	209 ± 22	
	C	Virginia (USA)	Coastal zone	Summer	D	67 ± 27	Caffrey & Kemp (1990)
	C	Virginia (USA)	Coastal zone	Autumn	D	99 ± 16	
	F	-	-	-	-	64.5	Flindt (1994)

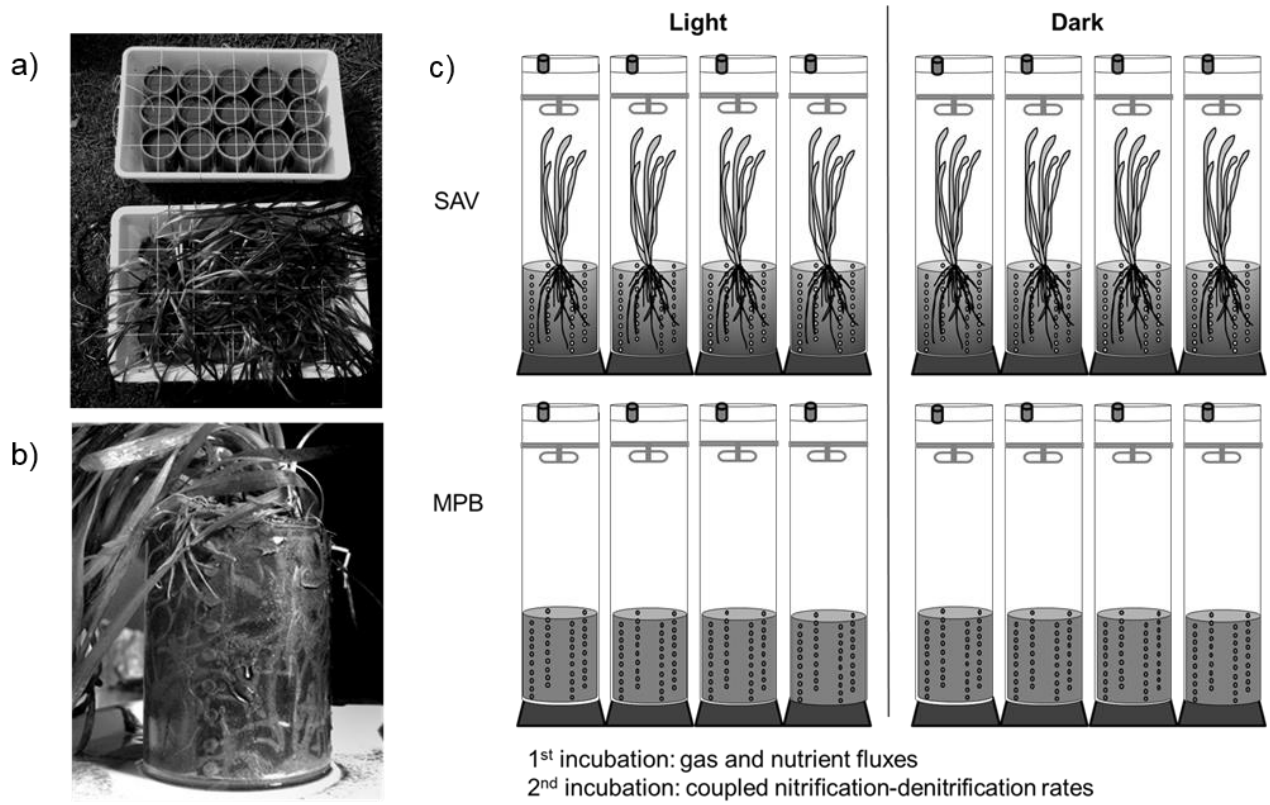
Figure captions

Fig. 1 At each site and sampling period 16 cylindrical microcosms were created with in situ sieved and homogenized sediments with (SAV, n=8) or without (MPB) shoots of *V. spiralis* (a). Microcosms were conditioned in situ for 3 weeks to allow plant growth and the development of microphytonbenthos and thereafter transferred underwater in transparent liners. After the conditioning period, the microcosms were transferred underwater in liners. Brownish halos were evident around root hair along the microcosm walls (b). For each site, the day after the recover, half microcosms were incubated in the light and half in the dark for TCO_2, $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ flux measurements (c). Thereafter, another incubation was performed after injection of $^{15}NH_4^+$ within sediments, to measure coupled nitrification-denitrification rates (see the text for more details)

Fig. 2 Light, dark and daily fluxes of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and DIN (dissolved inorganic N) measured seasonally in microcosms with SAV (*V. spiralis*) at M1 and M2 (average ± standard error, n=4)

Fig. 3 Light, dark and daily fluxes of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and DIN (dissolved inorganic N) measured seasonally in microcosms with MPB at M1 and M2 (average ± standard error, n=4)

Fig. 4 Light, dark and daily fluxes of coupled nitrification-denitrification rates (DNF_N) measured seasonally in microcosms with MPB at M1 and M2 (average ± standard error, n=4)



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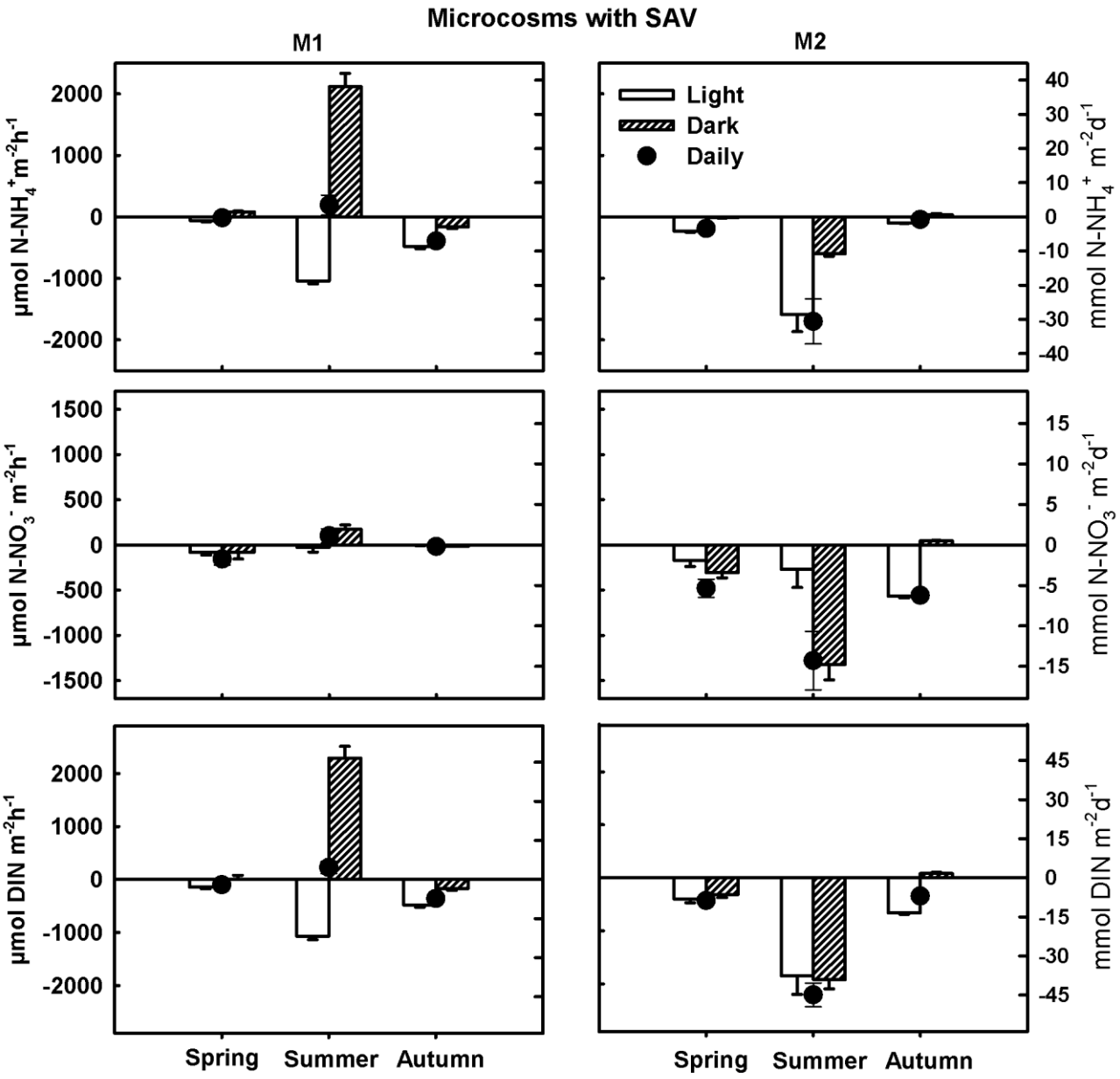

Fig. 2 Light, dark and daily fluxes of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and DIN (dissolved inorganic N) measured seasonally in microcosms with SAV (*V. spiralis*) at M1 and M2 (average ± standard error, n=4)

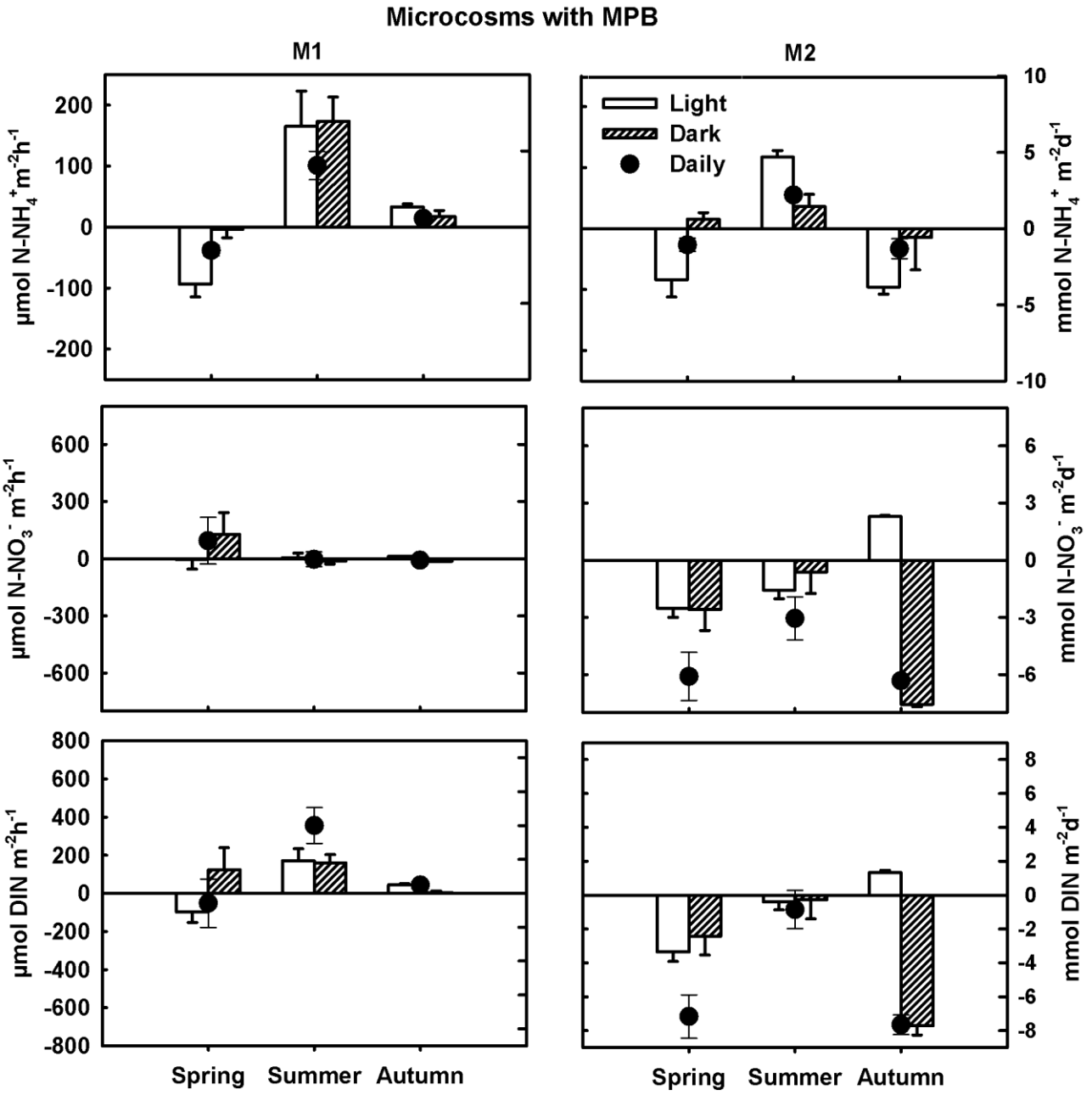

Fig. 3 Light, dark and daily fluxes of $N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and DIN (dissolved inorganic N) measured seasonally in microcosms with MPB at M1 and M2 (average ± standard error, n=4)

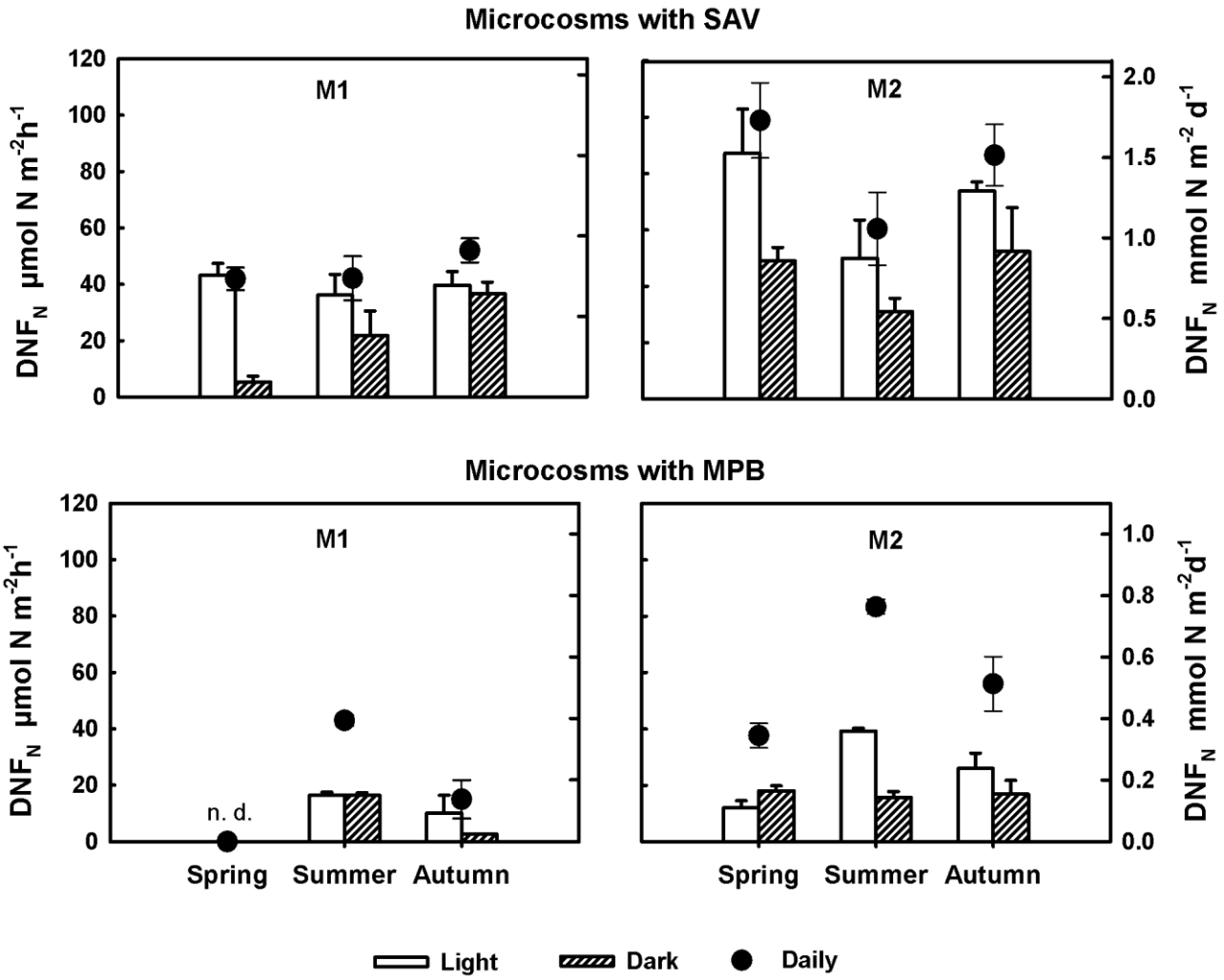

Fig. 4 Light, dark and daily fluxes of coupled nitrification-denitrification rates (DFN_N) measured seasonally in microcosms with MPB at M1 and M2 (average ± standard error, n=4)